UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,789,246
DATED : August 4, 1998
INVENTOR(S) : REID et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page,

Under item [62] Related U.S. Application Data, please substitute "which is a divisional of Ser. No. 741,128" to --which is a continuation of Ser. No. 741,128--.

Under Column 8-9 of the last page of the Patent, please insert claims 3-8 as follows:

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It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

--3. The composition of claim 1, wherein the extracellular matrix is formed from a material comprising collagen, fibronectin, laminin or combinations thereof.

--4. The composition of claim 3 wherein the collagen is type IV collagen.--

--5. The composition of claim 1 wherein the liver stromal cells are embryonic liver stromal cells.--

--6. The composition of claim 1 wherein the liver stromal cells are fetal liver stromal cells.--

--7. The composition of claim 1 which comprises a growth factor.--

--8. The genetically engineered hapatocyte precursor cells of claim 2 wherein the liver stromal cells are embryonic liver stromal cells or fetal liver stromal cells.--

Signed and Sealed this

Thirteenth Day of July, 1999

Attest:

Attesting Officer

Q. TODD DICKINSON

Acting Commissioner of Patents and Trademarks

(e.g., a polypeptide or a protein) of interest in biologically significant amounts. The hepatocyte precursors or the mature hepatocyte progeny therefrom, formed in this way can serve as a continuous drug delivery system to replace present regimens, which require periodic administration (by ingestion, injection, etc.) of the needed substance.

Genetically engineered hepatocyte precursors may be employed in the treatment of inherited disease and in the treatment of acquired disease. In the case of inherited diseases, this approach is used to provide genetically engineered hepatocyte precursors or mature hepatocytes differentiated therefrom, which contain DNA encoding a protein or polypeptide which an individual is unable to make correctly. Hepatocyte precursors of the present invention can also be used in the treatment of genetic diseases in which a product (e.g., LDL receptor) normally produced by the liver is not produced or is made in insufficient quantities. Here, hepatocyte precursors transduced with a DNA encoding the missing or inadequately produced substance can be used to produce it in sufficient quantities. In this case, at least a portion of the transduced hepatocyte precursors or a portion of their progeny differentiates into mature hepatocytes, which would produce LDL receptors and thus provide a means of preventing or treating familial hypercholesterolemia. This is an inherited disease in which the primary genetic defect is an abnormality in the expression or function of the receptor for low density lipoproteins, leading to elevated levels of serum cholesterol and the premature development of coronary artery disease. The transduced hepatocyte precursors, and the mature hepatocytes differentiated therefrom could be used to produce sufficient quantities of the LDL receptor to overcome the underlying defect. This approach may also be extended to any patient having a predisposition to atherosclerosis due to hyperlipidemia.

There are also acquired diseases for which treatment can be provided through use of genetically engineered hepatocyte precursors. The genetically engineered hepatocyte precursors may also be employed to treat viral hepatitis, particularly hepatitis B or nonA-nonB hepatitis, by gene transfer. For example, a gene encoding an anti-sense gene could be introduced into hepatocyte precursors to inhibit viral replication. In this case, a vector including a structural hepatitis gene in the reverse or opposite orientation would be introduced into hepatocyte precursors, resulting in production in the genetically engineered hepatocyte precursors and any mature hepatocytes differentiated therefrom of an anti-sense gene capable of inactivating the hepatitis virus or its RNA transcripts. Alternatively, the hepatocyte precursors may be transduced with a gene which encodes a protein, such as, for example, α-interferon, which may confer resistance to the hepatitis virus.

Advantages of employing hepatocyte precursors of the present invention include the provision of a model system for the growth of hepatocyte precursors and/or the differentiation of such hepatocyte precursors into mature hepatocytes. Such a model system of hepatocyte precursors has greater growth potential than cultures of mature hepatocytes, and thus is better suited for various studies of liver cells, such as toxicology studies, carcinogenic studies, and vaccine production. Also, because such hepatocyte precursors may be dissociated from liver tissue and then be enriched and expanded, such expanded hepatocyte precursors obtained from one liver may thus be administered therapeutically to a plurality of patients. The administration of such immature cells may also be less likely to stimulate immune rejection than the injection of mature hepatocytes. In addition, mature hepatocytes may have a limited life span and may undergo a limited number of cell divisions, whereas hepatocyte precursors have a greater capacity to generate daughter cells. Thus, the life span of such a system may be significantly prolonged and possibly may be indefinite.

Examples of non-therapeutic uses of hepatocyte precursors include research of liver embryology, liver cell lineages, and differentiation pathways; gene expression studies; mechanisms involved in liver injury and repair; research of inflammatory and infectious diseases of the liver; studies of pathogenetic mechanisms; and studies of mechanisms of liver cell transformation and etiology of liver cancer. Additional therapeutic uses include liver transplantation for patients with liver failure due to alcoholism, infection, congenital liver diseases, etc., gene therapy for liver diseases that are genetically based such as, for example, Wilson's disease, glycogen storage diseases, urea cycle enzyme defects, and Creigler-Najir disease; and the use of such hepatocyte precursors and any lineages of adult cells derived from them in assays for chemotherapy (eg., for liver cancers), for the production of vaccines for viruses that grow in the liver, and for studies of alcoholic cirrhosis. The hepatocyte precursors cells may also be employed as part of an "artificial liver;" i.e., the hepatocyte precursors may be placed in a container or apparatus, in which the hepatocyte precursors generate a liver lineage and function as a liver outside of the body. The container or apparatus is connected to the circulatory system of a human or animal subject.

In accordance with another aspect of the present invention, there is provided a composition comprising an animal cell population derived from liver. The cell population contains immature cells which are characterized by expression of alpha-fetoprotein or lack of essential expression of alpha-fetoprotein and albumin, and at least a portion of such cells or of the progeny of such cells is capable of differentiating into adult liver cells. The cells have been cultured under conditions which result in expansion of the immature cells. Such immature cells may be obtained from the livers of human or non-human animals hereinabove described. Although the progeny of such immature cells may differentiate into hepatocytes, such immature cells may differentiate into adult liver cells other than hepatocytes, such as bile duct cells, liver endothelial cells, and lipid-containing liver cells known as Ito cells.

Such immature cells derived from liver may be obtained from liver tissue and enriched or expanded under conditions hereinabove described for the enrichment and expansion of the above-described hepatocyte precursors. It is also contemplated that such immature cells may be genetically engineered through techniques such as those hereinabove described, whereby such genetically engineered cells may be administered to an animal or a human subject, in which the genetically engineered cells and/or their differentiated progeny express gene(s) of interest.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What is claimed is:

1. A composition comprising a cell culture of immature animal cells, including liver, pancreas, gut, lung, or bone marrow cells, which contain at least a population of hepatocyte precursor cells capable of differentiating into hepatocytes, serum-free culture medium, extracellular matrix and liver stromal cells.

2. Genetically-engineered hepatocyte precursor cells obtained by culturing immature animal cells, including liver,

ments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What is claimed is:

1. A composition comprising a cell culture of immature animal cells, including liver, pancreas, gut, lung, or bone marrow cells, which contains at least a population of hepatocyte precursor cells capable of differentiating into hepatocytes.

2. The composition of claim 1, further comprising extracellular matrix coated upon a porous solid support.

3. The composition of claim 2, wherein the solid support comprises Millicell membrane support, filters, sponges, and hollow fiber systems.

4. Genetically engineered hepatocyte precursor cells obtained by genetically engineering expanded hepatocytes precursor cells derived from culturing immature animal cells that contain at least a population of hepatocyte precursor cells capable of differentiating into hepatocytes.

5. The genetically engineered hepatocyte precursor cells of claim 4, wherein the hepatocyte precursor cells are differentiated into hepatocytes in a serum-free culture medium comprising extracellular matrix and liver stromal cells.

6. The genetically engineered hepatocyte precursor cells of claim 4, wherein the immature animal cells are selected from the group consisting of liver, pancreas, gut, lung, or bone marrow cells.

7. The genetically engineered hepatocyte precursor cells of claim 4, wherein the genetic engineering comprises ex vivo genetic modification of the hepatocyte precursors.

8. The genetically engineered hepatocyte precursor cells of claim 4, wherein genetically engineering comprises transfecting hepatocyte precursor cells with a vector comprising a genetic material that encodes polypeptides or protein of interest and/or a dominant selectable marker.

9. The genetically engineered hepatocyte precursor cells of claim 4, wherein the genetic material is under the control of retroviral vector regulatory elements and/or regulatory elements of genes normally expressed in the liver.

10. An isolated hepatocyte precursor capable of differentiating into a hepatocyte.

11. A cell culture comprising a population of the hepatocyte precursors of claim 10.

12. The cell culture of claim 11 further comprising extracellular matrix.

13. The cell culture of claim 12 in which the extracellular matrix comprises collagen, fibronectin, laminin, or combinations thereof.

14. The cell culture of claim 13 in which the collagen is used alone or in combination with proteoglycans or tissue extracts enriched in extracellular matrix materials.

15. The cell culture of claim 11 further comprising stromal cells.

16. The cell culture of claim 15 in which the stromal cells are liver stromal cells.

17. The cell culture of claim 15 in which the stromal cells are embryonic or fetal cells.

18. The cell culture of claim 11 further comprising a serum-free culture medium.

19. The isolated hepatocyte precursor of claim 10 which is obtained from a single cell suspension of liver cells.

20. The isolated hepatocyte precursor of claim 10 which is genetically engineered.

21. The genetically engineered isolated hepatocyte precursor of claim 20 in which the genetically engineered hepatocyte precursor expresses a protein or polypeptide.

22. The composition of claim 1 further comprising extracellular matrix formed from a material comprising collagen, fibronectin, laminin, or combinations thereof, wherein the collagen is used alone or in combination with proteoglycans or tissue extracts enriched in extracellular matrix materials.

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Genetically engineered hepatocyte precursors may be employed in the treatment of inherited disease and in the treatment of acquired disease. In the case of inherited diseases, this approach is used to provide genetically engineered hepatocyte precursors or mature hepatocytes differentiated therefrom, which contain DNA encoding a protein or polypeptide which an individual is unable to make correctly. Hepatocyte precursors of the present invention can also be used in the treatment of genetic diseases in which a product (e.g., LDL receptor) normally produced by the liver is not produced or is made in insufficient quantities. Here, hepatocyte precursors transduced with a DNA encoding the missing or inadequately produced substance can be used to produce it in sufficient quantities. In this case, at least a portion of the transduced hepatocyte precursors or a portion of their progeny differentiates into mature hepatocytes, which would produce LDL receptors and thus provide a means of preventing or treating familial hypercholesterolemia. This is an inherited disease in which the primary genetic defect is an abnormality in the expression or function of the receptor for low density lipoproteins, leading to elevated levels of serum cholesterol and the premature development of coronary artery disease. The transduced hepatocyte precursors, and the mature hepatocytes differentiated therefrom could be used to produce sufficient quantities of the LDL receptor to overcome the underlying defect. This approach may also be extended to any patient having a predisposition to atherosclerosis due to hyperlipidemia.

There are also acquired diseases for which treatment can be provided through use of genetically engineered hepatocyte precursors. The genetically engineered hepatocyte precursors may also be employed to treat viral hepatitis, particularly hepatitis B or nonA-nonB hepatitis, by gene transfer. For example, a gene encoding an anti-sense gene could be introduced into hepatocyte precursors to inhibit viral replication. In this case, a vector including a structural hepatitis gene in the reverse or opposite orientation would be introduced into hepatocyte precursors, resulting in production in the genetically engineered hepatocyte precursors and any mature hepatocytes differentiated therefrom of an anti-sense gene capable of inactivating the hepatitis virus or its RNA transcripts. Alternatively, the hepatocyte precursors may be transduced with a gene which encodes a protein, such as, for example, α-interferon, which may confer resistance to the hepatitis virus.

Advantages of employing hepatocyte precursors of the present invention include the provision of a model system for the growth of hepatocyte precursors and/or the differentiation of such hepatocyte precursors into mature hepatocytes. Such a model system of hepatocyte precursors has greater growth potential than cultures of mature hepatocytes, and thus is better suited for various studies of liver cells, such as toxicology studies, carcinogenic studies, and vaccine production. Also, because such hepatocyte precursors may be dissociated from liver tissue and then be enriched and expanded, such expanded hepatocyte precursors obtained from one liver may thus be administered therapeutically to a plurality of patients. The administration of such immature cells may also be less likely to stimulate immune rejection than the injection of mature hepatocytes. In addition, mature hepatocytes may have a limited life span and may undergo a limited number of cell divisions, whereas hepatocyte precursors have a greater capacity to generate daughter cells. Thus, the life span of such a system may be significantly prolonged and possibly may be indefinite.

Examples of non-therapeutic uses of hepatocyte precursors include research of liver embryology, liver cell lineages, and differentiation pathways; gene expression studies; mechanisms involved in liver injury and repair; research of inflammatory and infectious diseases of the liver; studies of pathogenetic mechanisms; and studies of mechanisms of liver cell transformation and etiology of liver cancer. Additional therapeutic uses include liver transplantation for patients with liver failure due to alcoholism, infection, congenital liver diseases, etc., gene therapy for liver diseases that are genetically based such as, for example, Wilson's disease, glycogen storage diseases, urea cycle enzyme defects, and Creigler-Najir disease; and the use of such hepatocyte precursors and any lineages of adult cells derived from them in assays for chemotherapy (e.g., for liver cancers), for the production of vaccines for viruses that grow in the liver, and for studies of alcoholic cirrhosis. The hepatocyte precursors cells may also be employed as part of an "artificial liver;" i.e., the hepatocyte precursors may be placed in a container or apparatus, in which the hepatocyte precursors generate a liver lineage and function as a liver outside of the body. The container or apparatus is connected to the circulatory system of a human or animal subject.

In accordance with another aspect of the present invention, there is provided a composition comprising an animal cell population derived from liver. The cell population contains immature cells which are characterized by expression of alpha-fetoprotein or lack of essential expression of alpha-fetoprotein and albumin, and at least a portion of such cells or of the progeny of such cells is capable of differentiating into adult liver cells. The cells have been cultured under conditions which result in expansion of the immature cells. Such immature cells may be obtained from the livers of human or non-human animals hereinabove described. Although the progeny of such immature cells may differentiate into hepatocytes, such immature cells may differentiate into adult liver cells other than hepatocytes, such as bile duct cells, liver endothelial cells, and lipid-containing liver cells known as Ito cells.

Such immature cells derived from liver may be obtained from liver tissue and enriched or expanded under conditions hereinabove described for the enrichment and expansion of the above-described hepatocyte precursors. It is also contemplated that such immature cells may be genetically engineered through techniques such as those hereinabove described, whereby such genetically engineered cells may be administered to an animal or a human subject, in which the genetically engineered cells and/or their differentiated progeny express gene(s) of interest.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What is claimed is:

1. A method of expanding hepatic precursor cells comprising culturing immature liver cells which contain at least a population of hepatic precursor cells in serum-free culture medium, an extracellular matrix and liver stromal cells such that hepatic precursor cells contained within the cell culture are expanded.

2. The method of claim 1 wherein the hepatic precursor cells are expanded at least three fold.

3. The method of claim 1 wherein the extracellular matrix is formed from a material selected from the group consisting of collagen, fibronectin, laminin, proteoglycan and combinations thereof.

4. The method of claim 5 wherein the collagen is Type IV.

5. The method of claim 1 wherein the liver stromal cells

are selected from the group consisting of embryonic liver stromal cells and fetal liver stromal cells.

6. The method of claim 1 wherein the culture further includes a growth factor.

7. A method of obtaining hepatic precursor cells comprising:

(a) culturing immature liver cells which contain at least a population of hepatic precursor cells in serum-free culture medium, an extracellular matrix and liver stromal cells such that hepatic precursor cells contained within the cell culture are expanded; and

(b) separating the expanded hepatic precursor cells from the culture medium so as to obtain hepatic precursor cells.

8. The method of claim 7 wherein the hepatic precursor cells are expanded at least three fold.

9. The method of claim 7 wherein the extracellular matrix is formed from a material selected from the group consisting of collagen, fibronectin, laminin and combinations thereof.

10. The method of claim 7 wherein the collagen is Type IV.

11. The method of claim 7 wherein the liver stromal cells are selected from the group consisting of embryonic liver stromal cells and fetal liver stromal cells.

12. The method of claim 7 wherein the culture further includes a growth factor.

13. A method of obtaining hepatic precursor cells comprising:

(a) culturing immature liver cells which contain at least a population of hepatic precursor cells in serum-free culture medium, an extracellular matrix, at least one growth factor, and liver stromal cells such that hepatic precursor cells contained within the cell culture are expanded; and

(b) separating the expanded hepatic precursor cells from the culture medium so as to obtain hepatic precursor cells.

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